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Ditylenchus africanus sp. n. from South Africa; a morphological and molecular characterization

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Summary – A new *Ditylenchus* species from South Africa that parasitizes peanut is described based on characteristics of morphology and restriction fragment length polymorphisms (RFLPs) of ribosomal DNA (rDNA). The new species, *Ditylenchus africanus* sp. n., is different from the two most similar species, *D. destructor* and *D. myceliophagus* by the following combination of characters : RFLP's generated by seven restriction enzymes on the internal transcribed spacer of rDNA, a medium long, relatively weak stylet (compared with the stylet of *D. destructor*), bursa length (as percentage of tail length) and spicule length.

Résumé – Ditylenchus africanus *n. sp. provenant d'Afrique du Sud; caractérisation morphologique et moléculaire* – Une nouvelle espèce de *Ditylenchus* parasite de l'arachide en Afrique du Sud est décrite en se fondant sur des caractères morphologiques et sur ceux provenant du polymorphisme des longueurs des fragments de restriction (RFLPs) de l'ADN ribosomal. La nouvelle espèce, *Ditylenchus africanus* n. sp., diffère des deux espèces les plus proches, *D. destructor* et *D. myceliophagus*, par la combinaison de caractères suivante : RFLPs provenant de sept enzymes de restriction situées sur l'espaceur interne transcrit de rADN, stylet de longueur moyenne et relativement peu robuste (en comparaison de celui de *D. destructor*), longueur de la bursa (exprimée en pourcentage de la longueur de la queue) et longueur des spicules.

Key words : *Ditylenchus*, morphology, systematics, ribosomal DNA, internal transcribed spacer, restriction fragment length polymorphisms, polymerase chain reaction.

Ditylenchus africanus sp. n. was first reported as *D. destructor* Thorne, 1945 from infested peanut fields, Schweizer-Reneke district, South Africa (Jones & De Waele, 1988). Since then this species has been found in several other areas of South Africa, attacking only peanuts and thriving at a temperature of 28 °C and higher (De Waele *et al.*, 1989). Populations of this nematode were tested on seven South African potato cultivars but no damage was done to the potato tubers and the potatoes were poor hosts (De Waele *et al.*, 1991). Consequently, the populations parasiting peanut were considered a distinct race of *D. destructor* with a limited host range (De Waele *et al.*, 1991). However, a molecular study of comparative taxonomy of some populations of *Ditylenchus* by Wendt (1992) threw doubt on this classification. Analysis of the ribosomal DNA (rDNA) of several geographic and host isolates of *D. dipsaci* (Kühn, 1857) Filipjev, 1936, *D. destructor* and *D. myceliophagus* Goodey, 1958 showed that the restriction fragment length polymorphisms (RFLP's) of the three species were distinct from each other (Wendt *et al.*, 1993). Moreover, digests of polymerase chain reaction (PCR) products showed that the *D. destructor* populations from South Africa differed from *D. destructor* isolates from the United Kingdom and the Wisconsin, USA (Wendt & Webster, 1992).

Fortuner (1982) showed that only a few characters are useful in differentiating *Ditylenchus* species, and so in addition to the light microscope study a comparative scanning electron microscope (SEM) study was done of the closely similar agricultural pest species of *Ditylenchus* using populations of *D. destructor, D. myceliophagus* and an isolate from South Africa. For comparison, a population of *D. dipsaci* was also studied. It is known that PCR technology is sufficiently sensitive to resolve differences between closely related populations. Consequently, this paper uses both morphological data, based on light and SEM observations, and molecular data to clarify the taxonomic status of the *Ditylenchus* population from peanut that has heretofore been regarded as *D. destructor*.

Materials and methods

ORIGIN AND CULTURE OF *DITYLENCHUS* POPULATIONS

The following nematode isolates were used :

Ditylenchus africanus sp. n. (DES SA) : Originally from hulls and seeds of infected peanuts from the Schweizer-Reneke district, South Africa; cultured on peanut callus tissue at the Grain Crops Institute, South Africa.

Ditylenchus destructor (DES UK) : Originally from potatoes in Ireland; cultured at Rothamsted Experimental Station, England and again at Simon Fraser University, Canada on *Rhizoctonia cerealis* plates.

Ditylenchus destructor (DES WIS) : Originally from potatoes in Wisconsin, USA; cultured on excised maize roots at the University of Wisconsin, Madison, USA and, subsequently, at Simon Fraser University.

Ditylenchus myceliophagus (MYC) : Originally from mushroom in Germany; cultured on fungal plates at Rothamsted Experimental Station and, subsquently, on *R. cerealis* at Simon Fraser University.

Ditylenchus dipsaci : Originally from teasel stems collected at Fivehead, Somerset, England; nematodes stored in dried tissue until required.

Microscopy

For SEM study, specimens were fixed in TAF, dehydrated in a graded ethanol series, critical point dried and coated with gold-palladium (25 nm). The specimens were viewed with a Jeol-35 scanning electron microscope at 15 kV.

For light microscopy, specimens were killed by the gradual application of heat, fixed in TAF and mounted in glycerine.

Molecular study

Ditylenchus nematodes were washed from the lids of fungal plates, or extracted from plant material using Baermann funnels. Nematode DNA was extracted from each population (Maniatis *et al.,* 1982; Webster *et al.,* 1990), and 100 to 200 ng samples were used for PCR amplifications of the ITS (internal transcribed spacer) region of ribosomal DNA, using primers as in Vrain *et al.* (1992), under conditions previously described (Wendt *et al.,* 1993). The amplified ITS fragment was digested with one of seven restriction enzymes, DdeI, HaeIII, HincII, HinfI, HpaII, PstI, and RsaI, following the manufacturer's recommendations (BRL, Ontario; Boerhringer Mannheim, Germany; Pharmacia, Québec). When necessary, the PCR product of several amplification reactions were pooled before digestion. DNA fragments were size fractionated by electrophoresis in agarose gel - 0.7 % to 1.5 % concentration, depending on the size of the fragments – with a 1 Kb ladder for markers (Pharmacia). Fragment sizes (Wendt *et al.,* 1993) and coefficients of dissimilarity (Vrain *et al.,* 1992) were calculated.

***Ditylenchus africanus* sp. n.**

= *D. destructor apud* De Waele *et al.,* 1989 (Figs 1-3)

Measurements *

Female (paratypes; n = 26) : L = 699-1140 (1014 ± 109.9) µm; a = 24.2-40.4 (29.3 ± 4.3); b = 7.1-11.8 (9.6 ± 1.2); b′ = 6.1-10.6 (8.8 ± 1.2); c = 8.8-16.9 (14.3 ± 1.9); c′ = 3.1-5.1 (3.9 ± 0.5); V = 77-81 (79.3 ± 1); G = 14-39 (21.4 ± 5.9) %; stylet length = 8-10 (8.9 ± 0.6) µm; cone length = 3.3-4 (3.6 ± 0.4) µm; shaft length = 4.5-6 (5.4 ± 0.4) µm; tail length = 55.5-82.5 (71.5 ± 6.4) µm. Dimensions on three fresh specimens : stylet length = 10.7-11.8 µm; cone length = 3.3-4.4 µm; shaft length = 7.4-7.7 µm.

Male (paratypes; n = 19) : L = 855-1014 (918 ± 46.9) µm; a = 31-42.4 (38.2 ± 4.6); b = 7.4-10.1 (8.5 ± 0.9); b′ = 5.9-9.2 (7.8 ± 0.9); c = 13-15.4 (14.0 ± 0.9); c′ = 3.3-5.8 (4.7 ± 0.6); T = 18.4-33 (25.2 ± 3.9) %; stylet length = 8-9.5 (8.7 ± 0.4) µm; cone length = 3-4 (3.4 ± 0.4) µm; shaft length = 4.5-6 (5.3 ± 0.4) µm; tail length = 53.5-75.5 (66 ± 5.7) µm; spicule length = 17-21 (19.8 ± 1.1) µm; gubernaculum length = 6-8 (7.4 ± 0.8) µm. Dimensions on one fresh specimen : stylet length = 10.5 µm; cone length = 4.5 µm; shaft length = 6 µm; spicule length = 24.5 µm.

Holotype (female). L = 970 µm; a = 32.3; b = 9.1; b′ = 8.8; c = 13.6; c′ = 3.6; V = 79;G = 23; stylet length = 8.5 µm; cone length = 3 µm; shaft length = 5.5 µm; tail length = 71.5 µm.

Description

Female : Head flattened, about 1.3 µm high and 6.4-7.3 µm wide, not offset from, but narrower than rest of body. SEM shows labial area with pore-like stoma opening surrounded by six outer labial sense organs and two large, medial lips, each with a pair of cephalic sensillae. Outline of labial area and head region hexagonal. Amphidial aperture elliptical, directed towards stomal opening. First head annule discontinuous, caused by position of amphidial apertures. Apart from labial disc, four lip annuli in lip region. Stylet delicate, knobs distinct, separated, sloping backwards; shaft about 60 % of total stylet length. Median bulb with crescentic valves. Basal bulb overlapping intestine. Postvulval uterine sac 50-143 (79.2 ± 21) µm long, comprising about 8 % of total body length or 37-85 % of vulva-anus distance and equal to 1.5-3.7 times vulval body diameter. Egg measurements : 45-60 µm × 20.5-33.5 µm. Tail elongate-conoid, tapering in posterior one-third to a finely rounded terminus.

Male : Bursa 33-60 (47 ± 8.6) µm long, leptoderan, covering 48-66 % of tail length. Spicule arcuate ventrad, slightly cephalated.

* For additional measurements, illustrations and descriptions see De Waele *et al.* (1989).

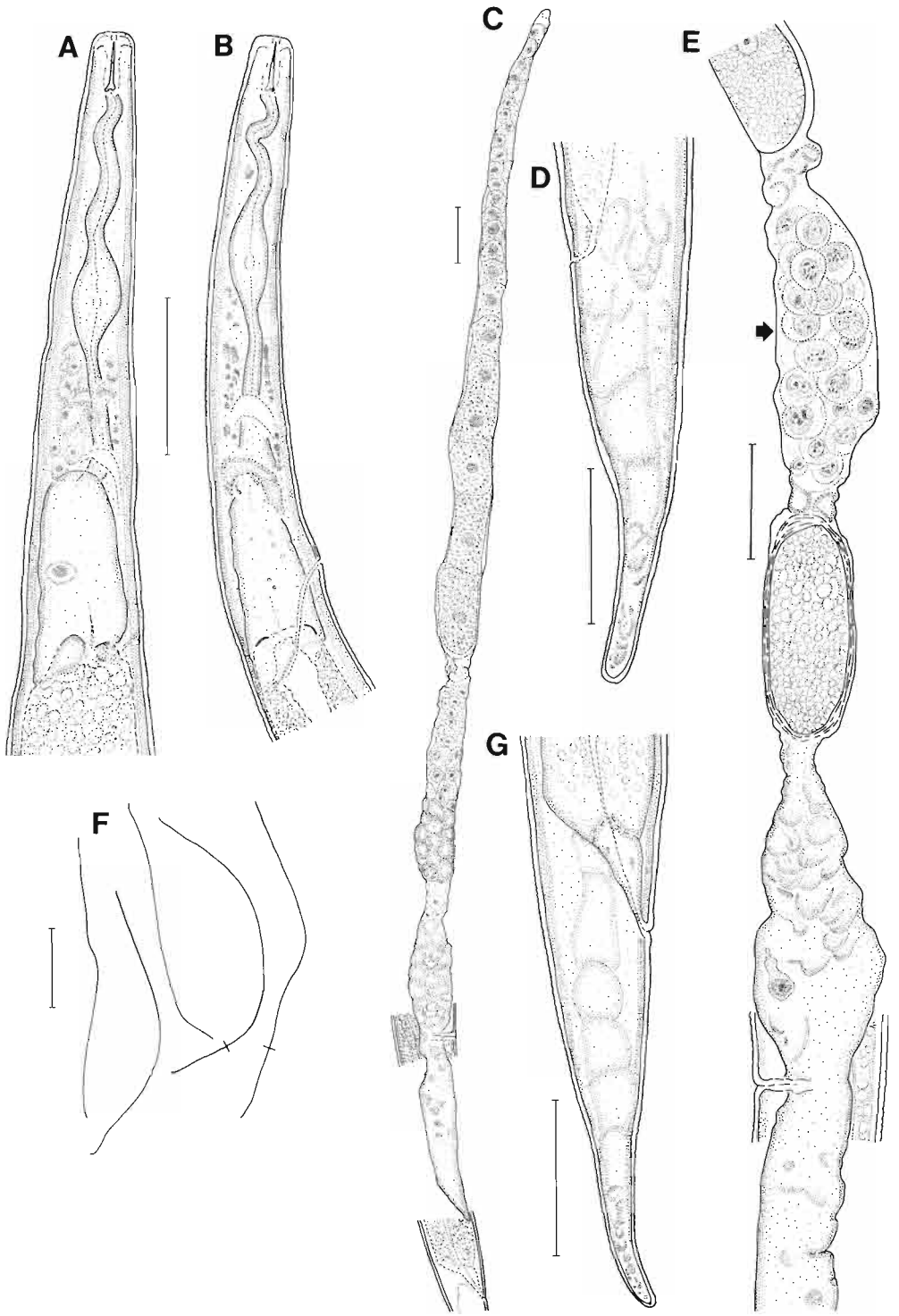

Fig. 1. Ditylenchus africanus *sp. n. A, B : Anterior region of female; C : Reproductive system of female; D : Female tail; E : Female reproductive system showing spermatheca with sperm (arrow); F : Male and female body postures; G : Female tail.* (Bar equivalents : A-E, G = 25 µm; F = 250 µm.)

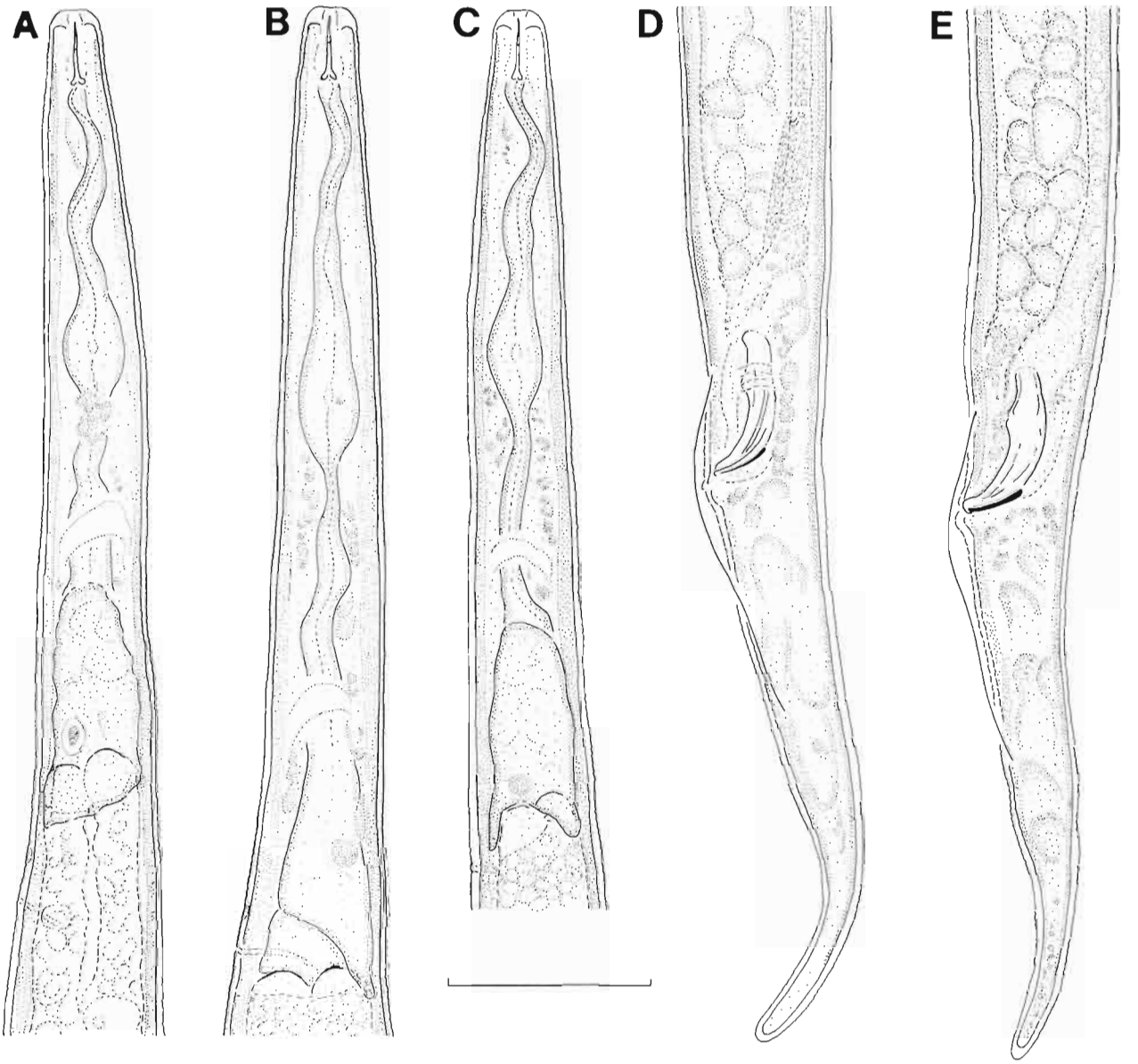

Fig. 2. Ditylenchus africanus *sp. n. A-C : Anterior region of male; D, E : Tail region of male.* (Bar equivalents 25 µm.)

TYPE SPECIMENS

Holotype female, 20 paratype females and fifteen paratype males on slides 27378-27385 are deposited in the National Collection of Nematodes, Plant Protection Research Institute, Pretoria, South Africa. Six paratype females and three paratype males are deposited at the Instituut voor Dierkunde, Rijksuniversiteit Gent, Ghent, Belgium. Three paratype females and seven paratype males are deposited at the Muséum National d'Histoire Naturelle, Paris, France.

TYPE LOCALITY AND HABITAT

Extracted from infected, discoloured peanut pods from rainfed peanut fields in the Schweizer-Reneke district, South Africa.

DIAGNOSIS

Relaxed body posture irregular. Lateral field with 6-15 lines. Outline of labial area hexagonal (SEM). Stylet delicate, 8-10 µm long, cone about 40 % and shaft 60 %

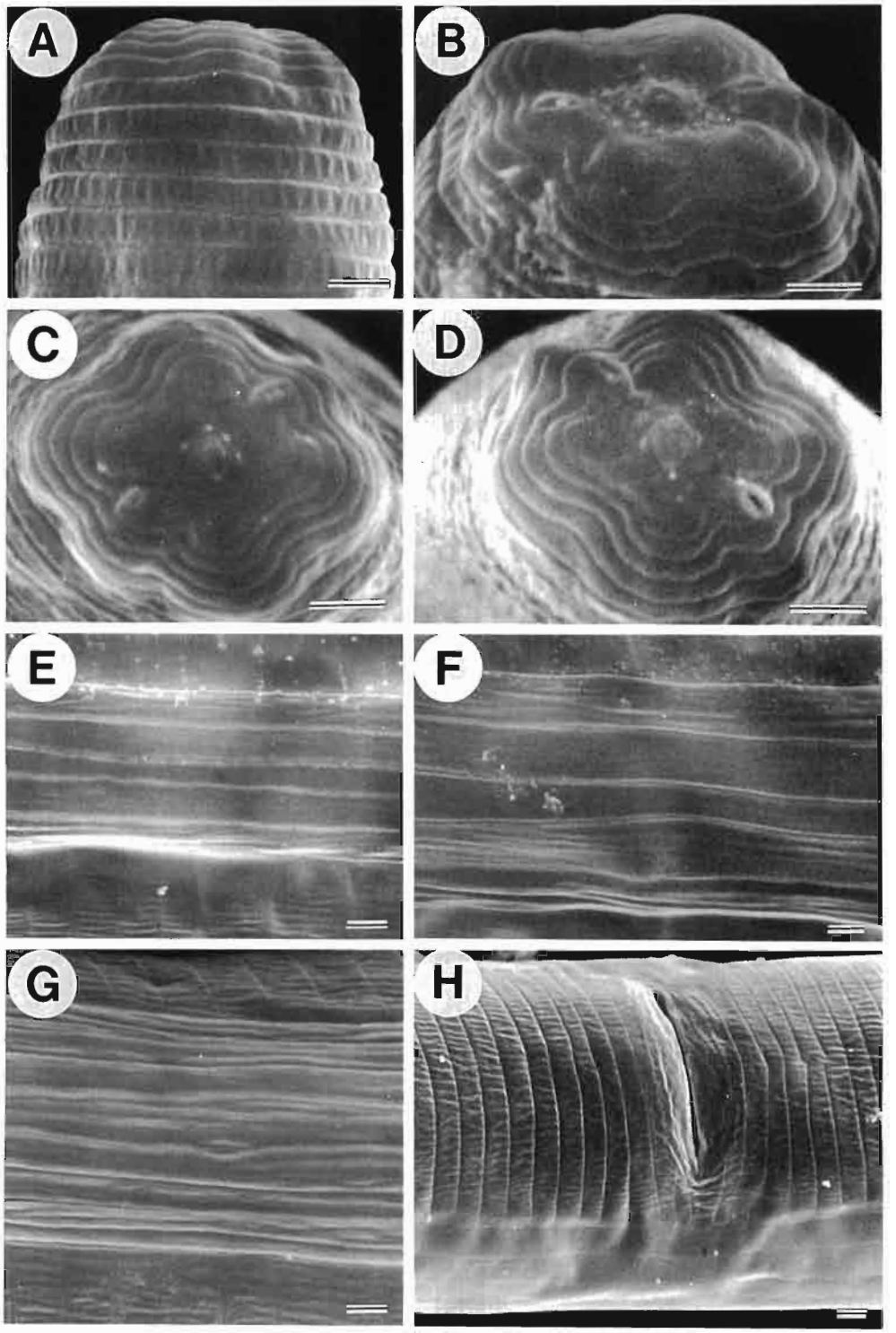

Fig. 3. Ditylenchus africanus *sp. n. A : Lateral view of female head; B : Female head; C, D : Frontal view of female head; E : Lateral field in anterior body region showing six longitudinal lines; F : Lateral field at about mid-body showing six longitudinal lines; G : Lateral field in posterior part of body showing about fifteen irregular longitudinal lines; H : Vulval area.* (Bar equivalents 1 μm.)

of total stylet length. Stylet knobs distinct, separated, sloping backwards. Median bulb with crescentic valves. Basal bulb overlapping intestine. Postvulval uterine sac long, 1.5-3.7 × vulval body diameter. Tail elongate conoid, tapering in posterior one third to a finely rounded terminus. Bursa covering 48-66 % of tail length.

RELATIONSHIPS

Ditylenchus africanus sp.n. is near to *D. anchilisposomus* (Tarjan, 1958) Fortuner, 1982; *D. clarus* Thorne & Malek, 1968; *D. destructor; D. longicauda* Choi & Geraert, 1988; *D. medians* (Thorne & Malek, 1968) Fortuner & Maggenti, 1987; *D. medicaginis* Wasilewska, 1965 and *D. myceliophagus*. It differs from *D. anchilisposomus* in the following: A slightly wider head (5.2-7.3 µm *vs* 5-6.5 µm); longer tail (53.5-82.5 µm *vs* 32-54 µm); slightly longer body (576-1140 µm *vs* 505-850 µm); well defined lateral lines, six to fifteen in number (six very faint lines in *D. anchilisposomus*). *D. africanus* sp.n. can be distinguished from *D. clarus* in the position of the excretory pore (opposite middle to opposite posterior end of basal bulb *vs* opposite middle of isthmus), position of hemizonid (within four annules anterior to excretory pore and just anterior to middle of basal bulb *vs* anterior and adjacent to excretory pore, just anterior to middle of isthmus) and position of nerve ring (around posterior part of isthmus *vs* forward, near median bulb). *D. africanus* sp. n. differs from *D. destructor* mainly in stylet length (8-10 *vs* 10-13 µm) : spicule length (15.4-22.1 *vs* 24-27 µm) and host preference (peanut *vs* range of host plants, except peanut). *D. africanus* sp.n. differs from *D. longicauda* in the following: Shorter tail (53.5-82.5 *vs* 88-122 µm) : higher c-value (8.8-16.9 *vs* 6.2-7.9) and lower c'-value (3.1-5.8 *vs* 7.1-12.1). *D. africanus* sp.n. can be distinguished from *D. medians* mainly by the longer stylet (8-10 *vs* 6.5-8 µm) and presence of crescentic valves in median bulb (absent in *D. medians*). The new species is different from *D. medicaginis* in the slightly lower c'-value (3.3-5.8 *vs* 4-8.6); bursa length as percentage of tail length (48-66 *vs* 20-44 %) and rounded tail tip (tail tip mostly pointed or dull in *D. medicaginis*). *D. africanus* sp. n. is very near *D. myceliophagus* but can be distinguished from this species mainly in the slightly longer stylet (8-10 *vs* 6.5-9 µm); bursa length as percentage of tail length (48-66 *vs* 20-55 %); cephalic framework (posterior blades very short, obscure *vs* posterior blades short, crescentic, refractive) and host preference (peanut *vs* cultivated mushroom).

The ITS region amplified by PCR of *D. africanus* was 1.0 Kb, while the WIS and UK isolates of *D. destructor* showed an ITS fragment of 1.2 Kb. The same ITS fragment was 0.9 Kb in both *D. myceliophagus* and *D. dipsaci*. The number and size of DNA restriction fragments generated by restriction of the ITS region of the WIS and UK isolates of *D. destructor* were identical using the seven restriction enzymes, but different from those of *D. africanus*. The number and size of restriction bands of *D. myceliophagus, D. dipsaci* and *D. africanus* were also different (Table 1). The proportion of bands shared by the species were small, and the coefficients of dissimilarity were quite high (Table 2).

Table 1. *Fragment sizes (base pairs) resulting from digestion of the ITS of* Ditylenchus africanus *n. sp.,* D. myceliophagus, D. destructor *(UK and WIS populations), and* D. dipsaci *with seven restriction enzymes. Fragments smaller than 100 bp were not included in this table.*

ENZYME	*D. dipsaci*	*D.destructor*	*D. africanus*	*D. myceliophagus*
DdeI		670		
		570		
	310			
	290		290	290
			250	250
	200		200	
HaeIII	900			
			650	
			540	
		450		450
				200
		170		
HincII			1000	
		900		900
	800			
		250		
HinfI		780		
				630
			450	
	440			
	350			
			310	310
		180		
	150		150	
		130	130	
			100	
HpaII		1000		
			950	
				900
	320			
	200			
	180			
PstI		850		
	650		650	
				620
			420	
	400	400		400
RsaI				900
			690	
		600		
	450		450	
	250	250		
		170		
	140			

Table 2. *Analysis of the restriction bands from Table 1 for four species of* Ditylenchus *showing (a) total number of restriction bands generated by seven restriction enzymes and the number of bands shared between species and (b) a matrix of coefficients of dissimilarity for these bands.*

(a) TOTAL NUMBER OF RESTRICTION BANDS GENERATED FOR EACH SPECIES (DIAGONAL) AND THE SHARED NUMBER OF BANDS BETWEEN SPECIES

	D. myceliophagus	*D. africanus*	*D. destructor*	*D. dipsaci*
D. myceliophagus	11	3	3	2
D. africanus		16	1	6
D. destructor			15	2
D. dipsaci				16

(b) COEFFICIENTS OF DISSIMILARITY

	D. myceliophagus	*D. africanus*	*D. destructor*	*D. dipsaci*
D. myceliophagus	000	.778	.769	.852
D. africanus		000	.935	.625
D. destructor			000	.871
D. dipsaci				000

DISCUSSION

The genus *Ditylenchus* shows little intragenic diversity in most of the morphological characters but there is substantial intraspecific variation which makes identification difficult (Sturhan & Brzeski, 1991). Only a few of the morphological characters are sufficiently consistent to be useful in identification (Fortuner, 1982), namely the number of lines in the lateral field, shape of tail terminus, c', c (in some species), stylet length, length of postuterine sac expressed in vulval body diameters (only in a broad sense), V-value (most species identical) and spicule and bursa length. On the basis of these morphological characters *Ditylenchus africanus* sp. n. is very similar to *D. myceliophagus*. However, it differs significantly from *D. myceliophagus* in its molecular character as well as in its host specificity. *D. myceliophagus,* normally considered fungivorous, has been observed in rice panicles and sorghum root but could not be proven to feed and reproduce on these higher plants (Sturhan & Brzeski, 1991).

Morphologically *D. africanus* sp. n. is very close to both *D. destructor* and *D. myceliophagus,* and De Waele *et al.* (1991) considered it to be a race of *D. destructor* with a limited host range. Our SEM data (Figs 3, 5, 6) on the external morphology tends to reinforce the similarity between these members of the genus *Ditylenchus*. Therefore, only through a technique as sensitive as RFLP analysis has it been possible to clearly differentiate the three species. The size (and probably structure) of the ITS region, amplified between the primer's sequences in the PCR reaction, was different for the three plant parasitic species examined (*D. dipsaci, D. destructor* and *D. africanus*), but the two populations of *D. destructor* had an ITS region of the same size. Almost all restriction sites in the ITS of the DES SA population *(D. africanus)* were unique and were not found in the other species examined. Wendt *et al.* (1993) found no RFLPs in the ITS region of seven races of *D. dipsaci* that could not be distinguished from each other using this technique, but these *D. dipsaci* races could all be easily distinguished, solely on the basis of RFLP data, from

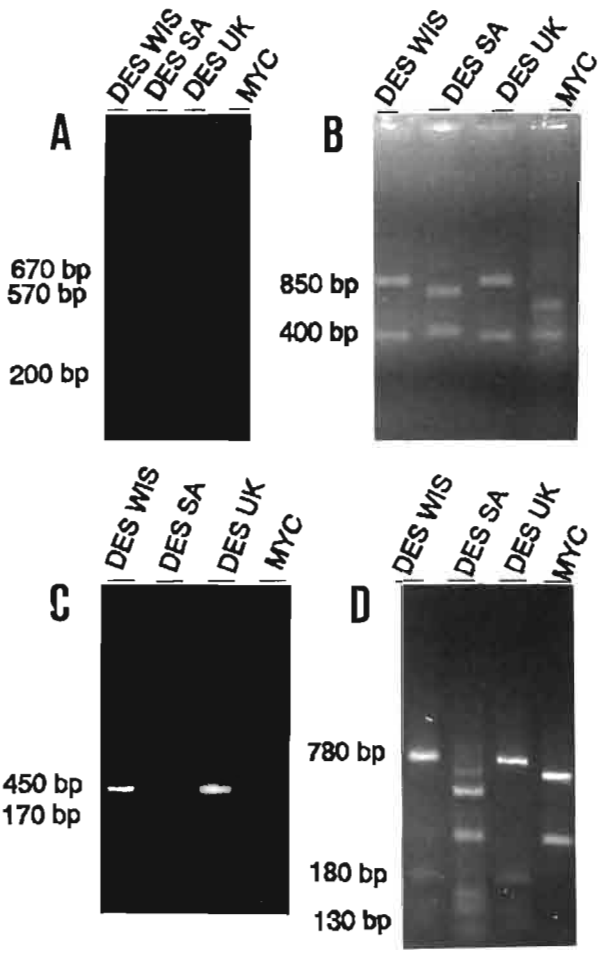

Fig. 4. *Agarose gels of the PCR amplified spacer ITS of United States, Wisconsin (DES WIS) and United Kingdom (DES UK) populations of* Ditylenchus destructor, D. africanus sp. n. (DES SA) from South Africa and *D. myceliophagus (MYC). A : Digested with the restriction enzyme* Dde1; *B : Digested with the restriction enzyme PstI; C : Digested with the restriction enzyme* HaeIII; *D : Digested with the restriction enzyme* HinfI.

Faint bands larger than the strong 450 bp band in DES SA result from a partial digest. (Marker = 1 Kb.)

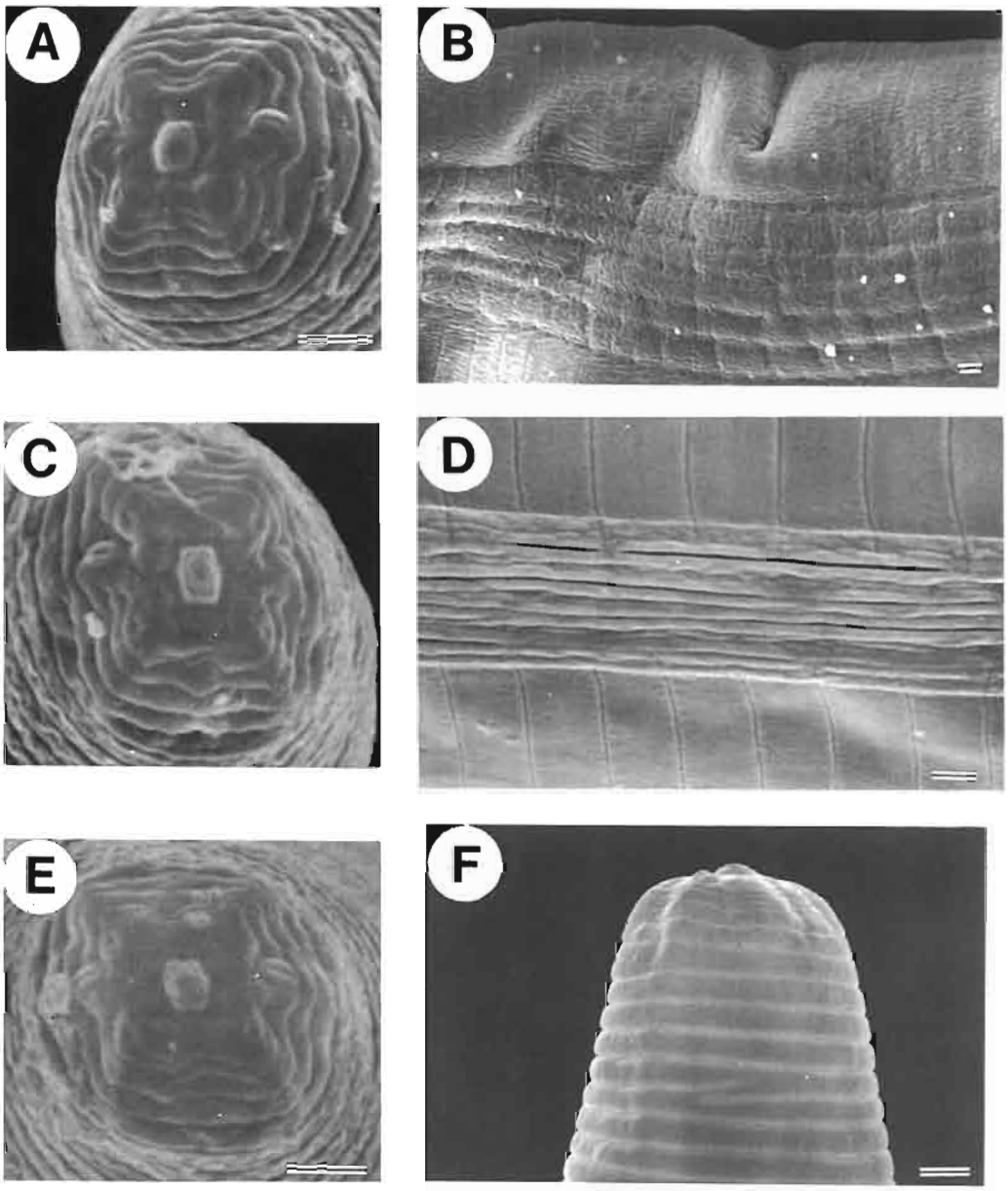

Fig. 5. Ditylenchus myceliophagus. *A : Frontal view of female head; B : Lateral field showing six longitudinal lines; C : Frontal view of female head; D : Lateral field showing about eleven lines; E : Frontal view of female head; F : Lateral view of female head region.* (Bar equivalents = 1 μm.)

both *D. destructor* and *D. myceliophagus*. Ferris *et al.* (1993) in their analysis of the ITS region of cyst-forming nematodes found that *Heterodera glycines, H. schachtii* and *H. trifolii* had fewer than five base pair differences in ITS1 and ITS2 sequences. The RFLP data presented here (Fig. 4), shows that the ITS regions of four species of *Ditylenchus* are much more variable than that of the cyst-forming species. Although the sample examined was small, the RFLP data presented here leaves no doubt that DES SA is well separated from *D. myceliophagus, D. dipsaci* and *D. destructor,* and that it should not be considered a race or a sibling of any of these species.

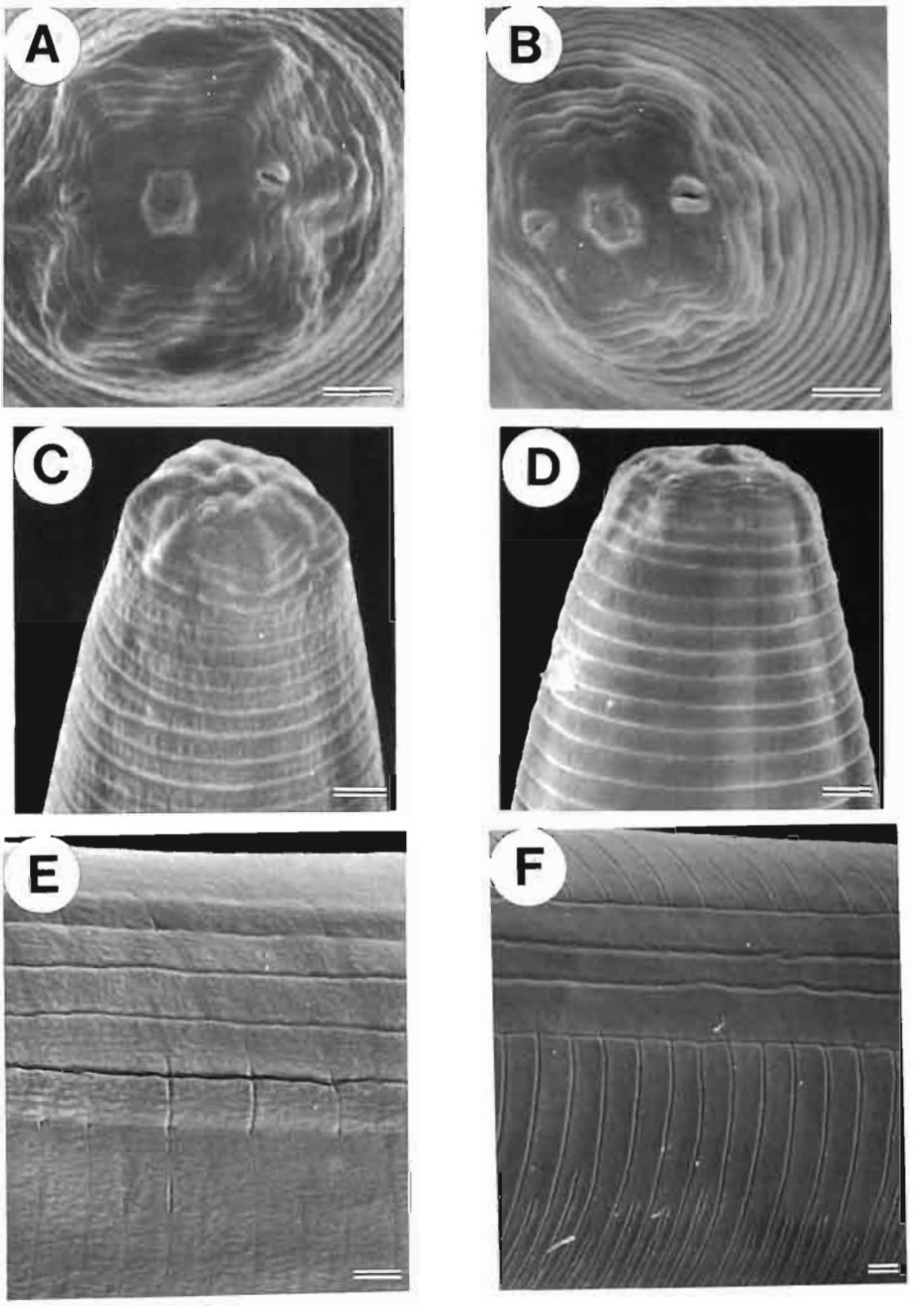

Fig. 6. Ditylenchus destructor. *A : Frontal view of female head; C : Female head; E : Lateral field showing six longitudinal lines.* – D. dipsaci. *B : Frontal view of female head; D : Lateral view of female head; F : Lateral field showing four lines.* (Bar equivalents = 1 µm.)

SEM observations on *Ditylenchus* spp

DITYLENCHUS AFRICANUS sp. n. (Fig. 3 A-H)

Labial area hexagonal in outline, medial lips large, each indented to form two submedial lobes, each lobe with a cephalic sense organ. Amphidial apertures situated laterally on first and second annules, causing the first annule to be discontinuous in contour. Lateral field with six lines, becoming subdivided to a maximum of fifteen lines.

D. MYCELIOPHAGUS (Fig. 5 A-F)

Labial area hexagonal in outline, medial lips large, each indented to form two submedial lobes. Amphidial aperture situated laterally on first, second and sometimes third head annules, disrupting the contours of both first and second head annules. Lateral field with six lines, in some specimens becoming subdivided to a maximum of twelve lines.

D. DESTRUCTOR (Fig. 6, A, C, E)

Labial area hexagonal to squarish in outline, medial lips large, subdivided into submedial lobes by a small bulging area. Amphidial apertures situated laterally on first and second head annules, disrupting the contours of both annules. Lateral field with six lines.

D. DIPSACI (Fig. 6, B, D, F)

Labial area squarish in outline, medial lips large, each lip subdivided into two submedial lobes by a small, medial bulging area. Amphidial apertures situated virtually on labial area, disrupting contour of first head annule only. Lateral field with four lines.

The stomal opening of all specimens is pore-like, surrounded by six labial papillae. The head area is difficult to define but according to the hexagonal contours of the annules overlying the cephalic framework, the lip region is comprised of the labial disc and four head annules in all populations studied.

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